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Theory of conformational changes in supercoiled DNA

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Abstract : A closed circular DNA molecule is constrained by the invariance of linking number of its two strands as long as the molecule maintains its closed form. In a negatively supercoiled DNA, which applies to the usual plasmids occurring in nature, such as pBR 322, ColE1 or ϕX174, this linking number Lk is lower than that of its relaxed form, namely Lk_0. The resulting linking difference $\Delta Lk = (Lk - Lk_0)$ of the closed circular molecule is distributed linearly between a change in its duplex twist ΔTw and an axial writhe Wr. The stress imposed by these elastic deformations may be relieved at the cost of local conformational changes introduced within the topologically constrained molecule, such as the B-Z, cruciform or melting transition, as the case may be. The cooperativity of such transitions and the other significant features, which are under detailed theoretical investigations by our group, have been reviewed and analysed in the light of the available experimental data.

Keywords : Supercoiled DNA, linking number conservation, conformation of molecules.

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1. Introduction

It is well known that a covalently closed circular DNA duplex is constrained by the fact that its linking number Lk is conserved as long as the molecule maintains its closed form. In a negatively supercoiled or underwound DNA, which is the case for all known plasmids found in nature, this linking number Lk is lower than that of its relaxed form, namely Lk_0. The resulting linking difference $\Delta Lk = (Lk - Lk_0)$ of the closed circular molecule is distributed linearly between a change in its duplex twist ΔTw and an axial writhe Wr (Crick 1976), namely

$$\Delta Lk = \Delta Tw + Wr \tag{1}$$

The aim of this review is to analyse how the stress imposed by these elastic deformations may be relieved at the cost of local conformational changes introduced within the topologically constrained molecule. Thus we obtain a general expression for the supercoiling energy G_s in terms of the elastic parameters of a partially transformed closed circular DNA having an initial linking difference ΔLk. Then we take up the specific examples of structural transformations, such as the B-Z

transition where an appropriate purine-pyrimidine stretch exists in the molecule, the transition to a cruciform state where a suitable palindromic sequence exists, and finally the melting transition which causes the presence of denatured regions in the molecule.

It is generally believed that each of the above mentioned transitions in DNA, which represents a possible excursion from its usual Watson-Crick form or the B form, plays a significant role in molecular regulatory processes. In the present work, we apply thermodynamical considerations to show that these transitions are indeed facilitated in a negatively supercoiled molecule and are, therefore, clearly feasible under physiological conditions. The results of these investigations are analysed in the light of experimental data, wherever available.

2. Supercoiling energy

Let us suppose that a supercoiled B DNA molecule, N base pairs long, has an initial linking difference ΔLk or a supercoil density $\sigma = \Delta Lk/(N/A_B)$, where $A_B = 10.4$ is the number of base pairs per helical turn of the molecule. The total linking number for such a molecule is then given by

$$Lk = (N/A_B) + \Delta Lk \tag{2}$$

where $N/A_B = Lk_0$ represents the linking number of its relaxed form. Let us further suppose that n base pairs within the molecule have actually been driven into a transformed conformation T having A_T base pairs per turn. Then, for this partially transformed molecule, the total linking number is given by

$$\Delta Lk_T = [(N-n)/A_B) \pm (n/A_T) + \Delta Lk_T \tag{3}$$

where ΔLk_T denotes the residual linking difference after n base pairs have actually been transformed in the molecule, and the $\pm$ sign refers to the handedness of the transformed helical region. Conservation of the total linking number gives $Lk_T = Lk$, so that combining eqs. (2) and (3), we get

$$\Delta Lk_T(\sigma, n) = \Delta Lk + n\left(\frac{1}{A_B} \pm \frac{1}{A_T}\right) \tag{4}$$

where $\Delta Lk = (N/A_B)\sigma$, σ being the supercoil density. The positive sign applies to a transformation which involves a change in the handedness of the transformed helical region, while the negative sign applies when there is no such change. Proceeding along the same lines as in our earlier paper (Sen and Majumdar 1988), the supercoiling energy of the partially transformed molecule is then given by

$$G_s(\sigma, n) = 2\pi^2 C_B[\Delta Lk_T(\sigma, n)]^2/[N + (\kappa - 1)n] \tag{5}$$

where $\kappa = C_B/C_T$, C_B and C_T being the average torsional stiffness constants of the B region and the transformed region respectively. In obtaining this expression for supercoiling energy, only the contribution arising from torsional deformations has been taken into account, ignoring the effect of axial writhe, assumed to be

generally small. Nevertheless, it can be shown that the writhing component does not alter the functional form of eq. (5), but only redefines the constants κ and C_B, so as to include the elastic bending parameters as well (Sen and Lahiri 1991). This, however, does not effect our conclusions as the values used by us for these constants have been obtained by fitting the experimental data. Furthermore, it has also been shown in the same paper that the transition in a closed molecule is rather insensitive to its base content, so that we are perfectly justified in treating it as a homopolymer with average elastic parameters.

3. B-Z Transition

Let us assume that the closed circular B DNA has a Z-transformable purine-pyrimidine sequence, n_0 base pairs long, out of which a stretch of n base pairs has actually been driven into the left-handed Z form with $A_T \equiv A_Z = 12.0$ base pairs per turn. As the transformed part of the molecule is also an ordered helical structure, we assume that the torsional stiffness constants $C_Z = C_B = C$, so that $\kappa = 1$. Under this condition, the eq. (5) for the supercoiling energy is reduced to the form

$$G_S(\sigma, n) = 2\pi^2 C[\Delta Lk_Z(\sigma, n)]^2/N \tag{6}$$

where, according to eq. (4),

$$\Delta Lk_Z(\sigma, n) = \Delta Lk + n\left(\frac{1}{A_B} + \frac{1}{A_Z}\right) \tag{7}$$

and $\Delta Lk = (N/A_B)\sigma$. This clearly shows that, in the present case, the supercoiling energy G_S is always proportional to $(\Delta Lk_Z)^2$ for the transforming molecule.

Assuming that the n transformed base pairs are distributed into n_r regions, the total free energy of the supercoiled molecule, for a given configuration, may be expressed in the form

$$E_Z(\sigma, n, n_r) = G_S(\sigma, n) + n_r G_r + nG_{BZ} \tag{8}$$

where G_r is the average nucleation energy per region of the transformation (which is equal to twice the junction energy G_j) and G_{BZ} is the average change in intrinsic free energy per base pair arising out of the structural changes involved. The partition function of the chain molecule is then given by

$$Z = \sum g e^{-E_Z/kT} \tag{9}$$

where k is the Boltzmann constant and T is the absolute temperature. The degeneracy factor g represents the number of ways in which the n transformed base pairs can be distributed into n_r regions of the Z-transformable stretch, n_0 base pairs long, and is given by

$$g(n_0, n, n_r) = \frac{(n-1)!(n_0-n+1)!}{(n-n_r)!(n_0-n-n_r+1)!(n_r-1)!n_r!} \tag{10}$$

Therefore, the average number of base pairs $\langle n \rangle$ in the left-handed Z conformation is given by

$$\langle n \rangle = \frac{1}{Z}\left[\sum_{n,n_r} n g e^{-E_Z/kT}\right] \tag{11}$$

whence the transformed fraction $\theta = \langle n \rangle / n_0$, as a function of the supercoil density σ, may be computed at any given temperature T.

We have applied the above method to investigate the nature of the supercoil-induced B-Z transition in the purine-pyrimidine sequence $d(GT)_{30}.d(AC)_{30}$, cloned into a specially constructed cloning vector (pDPL6) containing the origin of replication and ampicillin resistance gene of pBR322. The transition curve, as shown in Figure 1, is obtained for appropriate values of the elastic and the energy parameters, namely $C = 2.5 \times 10^{-19}$ erg/rad^2, $G_r = 15.4$ kcal/mole and $G_{BZ} =$

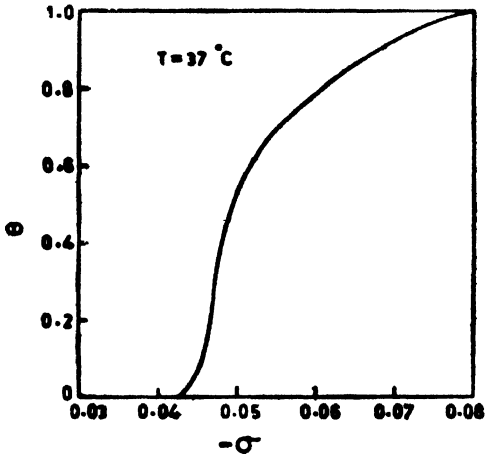

Figure 1. Supercoil-induced B-Z transition curve in a purine-pyrimidine sequence indicating cooperative effect at critical supercoil density $\sigma = -0.047$.

0.45 kcal/mole. The mid-point of the sigmoidal portion of the transition curve (Figure 1) gives the critical supercoil density $\sigma = -0.047$ at which the cooperative part of the B-Z transition sets in at the physiological temperature, namely at $T = 37°C$. This result is in perfect agreement with corresponding experimental value for σ (Haniford and Pulleyblanck 1983). For the present length of the purine-pyrimidine sequence considered, namely $n_0 = 60$, the cooperative part of the B-Z transition is followed by a subsequent elongation of the Z-stretch, as shown in Figure 1, until the transition is complete. These preliminary results, based on statistical mechanical considerations, are clearly an improvement over our earlier work (Sen and Majumdar 1987), where the transition curve was obtained through a mere energy minimisation procedure. Further details, along these lines, for the above B-Z transition will be published elsewhere (Majumdar *et al* 1991).

4. Transition to cruciform states

For transition to cruciform states in a supercoiled B DNA having palindromic or inverted repeat sequences, we obtain features similar to those in the case of *B-Z* transition. Let us assume that an inverted repeat sequence having n_0 base pairs exists in the supercoiled molecule out of which a stretch of n base pairs is driven into a cruciform state, where each of the two constituent hairpins consists of m base pairs at the stem and p denatured bases at the loop. Then, in the whole molecule, $n=(2m+p)$ base pairs will not consume any linking number, as the two hairpins are formed out of separate strands of the closed circular DNA duplex. The supercoiling energy of the molecule in this configuration is given by

$$G_s(\sigma, m)=2\pi^2 C_B[\Delta Lk_+(\sigma, m)]^2/[N-(2m+p)] \tag{12}$$

where

$$\Delta Lk_+(\sigma, m)=\Delta Lk+(2m+p)/A_B \tag{13}$$

and $\Delta Lk=(N/A_B)\sigma$. These are obtained from eqs. (4) and (5) by putting $A_T \equiv A_+=\infty$ for the cruciform state, as the two strands initially forming the helical turns with $A_B=10.4$ are now separated from each other, and $\alpha=0$. The latter condition implies that the torsional rigidity parameter $C_+=\infty$ for the extruded cruciform, as this region is considered to be rigid or undeformable in comparison with the rest of the molecule. Hence the total energy of the transforming molecule is given by

$$E_+(\sigma, m)=G_s(\sigma, m)+\epsilon_L \tag{14}$$

where ϵ_L represents the "loop energy" or the nucleation energy for cruciform extrusion, involving the energy of melting of the p base pairs and that of bending of the single strand in the loops. Clearly, as the stems of the cruciform with $2m$ base pairs involve the same helical structure as in the usual B DNA, no other energy parameter is involved in the process. Hence, for the partition function, we have

$$Z=\sum_m e^{-E_+(\sigma, m)/kT} \tag{15}$$

Therefore, the average number of the transformed base pairs at any given temperature is given by

$$\langle n\rangle=\frac{1}{Z}\sum_m n e^{-E_+(\sigma, m)/kT} \tag{16}$$

where $n=(2m+p)$, so that the transformed fraction $\theta=\langle n\rangle/n_0$ may be computed as a function of the supercoil density at the physiological temperature ($T=37°C$). The supercoil-induced cruciform transition curve is plotted in Figure 2 with the

average energy parameter $\epsilon_L = 1.3 \times 10^{-12}$ erg/loop for a 31 base pair long inverted repeat of ColE1 inserted into the supercoiled plasmid DNA molecule pColIR515 containing 2740 base pairs including the insert. For the present length of the

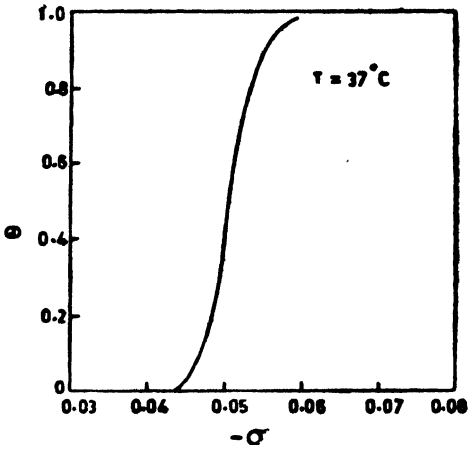

Figure 2. Supercoil-induced cooperative transition of an inverted repeat sequence into a cruciform state at critical supercoil density $\sigma = -0.051$.

inverted repeat sequence, namely $n_0 = 31$, we find that the entire transition is cooperative at the critical supercoil density $\sigma = -0.051$ (Figure 2), which agrees very well with the corresponding experimental data (Lilley and Hallam 1984).

5. Melting transition

The melting of B DNA involves snapping of hydrogen bonds, leading to the transition from an ordered helical state with $A_B = 10.4$ into the random coil, for which $A_T \equiv A_C = \infty$. Also, in the present case, the torsional rigidity parameters $C_B \gg C_C$, so that $\alpha \gg 1$. Hence, from eqs. (4) and (5), the supercoiling energy associated with the molecule, N base pairs long, out of which n base pairs are melted, is given by

$$G_s(n, \sigma) = 2\pi^2 C_B [\Delta Lk_0(n, \sigma)]^2 / [N + (\alpha - 1)n] \tag{17}$$

where

$$\Delta Lk_0(n, \sigma) = \Delta Lk + (n/A_B) \tag{18}$$

and $\Delta Lk = (N/A_B)\sigma$. Thus, in the present case, it is important to note that the supercoiling energy G_s is no longer proportional to $(\Delta Lk_0)^2$, as in the case of the cruciform transition, but unlike what happens for the B-Z transition. Combining eqs. (17) and (18), we get

$$G_s(\theta, \sigma) = 2\pi^2 C_B N(\theta + \sigma)^2 / [1 + (\alpha - 1)\theta] A_B^2 \tag{19}$$

where $\theta = n/N$ denotes the fraction of base pairs melted, and σ is the supercoil density. Let us assume that the n melted base pairs and the $(N-n)$ helical ones

in the closed circular DNA are distributed into n_r melted regions and n_r helical regions respectively. Then $n_j = 2n_r$ represents an even number of junctions formed within the melting molecule in its closed circular form. Therefore, the total free energy of the molecule is given by

$$E_\sigma(\sigma, n, n_r) = G_\beta(\sigma, n) + n(\epsilon - T\Delta S) + n_r\epsilon_0(1-\delta_{n0})(1-\delta_{nN}) \tag{20}$$

where $(\epsilon - T\Delta S)$ represents the free energy of a broken base pair, and ϵ_0 is the nucleation energy for melting which, in the present case, is equal to the DNA stacking energy (Sen and Majumdar 1988). The partition function of the closed chain molecule is then given by

$$Z = \sum_{n, n_r} g e^{-E_\sigma/kT} \tag{21}$$

where the degeneracy factor g represents the number of ways in which the n melted pairs and the $(N-n)$ helical ones may be distributed into n_r melted regions and n_r helical regions respectively, and is given by

$$g(N, n, n_r) = \frac{N(n-1)\,!\,(N-n-1)\,!}{(n-n_r)\,!\,(N-n-n_r)\,!\,(n_r-1)\,!\,n_r\,!} \tag{22}$$

This follows from an extension of the usual degeneracy factor for a linear chain system to the closed chain system (Sen and Majumdar 1988).

As the degree of polymerisation N for supercoiled DNA is large enough, and the transition can occur anywhere within the entire circular stretch of N base pairs, we can only evaluate the partition function Z by approximating the summation of eq. (21) by its largest term. The usual technique in such cases is to optimise $\ln [g.\exp(-E_\sigma/kT)]$ with respect to n and n_r for the partially melted molecule, use Stirling's approximation to simplify the various terms involved, and finally eliminate n_r from the resulting equations. We thus obtain

$$2\eta\theta(1-\theta)(\xi-1) - [(1-\theta)\xi - \theta][1 - \{1 - 4\eta\theta(1-\theta)\}^{\frac{1}{2}}] = 0 \tag{23}$$

where

$$\xi = \exp\left[\left(\epsilon - T\Delta S + \frac{\partial G_\beta}{\partial n}\right)\Big/ kT\right] \tag{24}$$

and

$$\eta = 1 - \exp(\epsilon_0/kT) \tag{25}$$

where $\Delta S = 12.0$ e.u. represents the change in conformational entropy per base pair and its value can be estimated by considering the number of flexible bonds involved in a pair of nucleotides (Majumdar and Pathria 1985, Majumdar and Thakur 1985). By choosing appropriate values for the average elastic and energy parameters in an earlier work (Sen and Majumdar 1988), the eq. (23) was solved iteratively by Newton-Raphson method to obtain the thermal denaturation curve (θ vs T) for the

ϕX174 plasmid DNA having $\sigma = -0.06$. The results were compared with the observed melting profile for ϕX174 in tetraethylammonium bromide (TEA) solution, where the molecule behaves like a homopolymer (Gagua *et al* 1981), and the

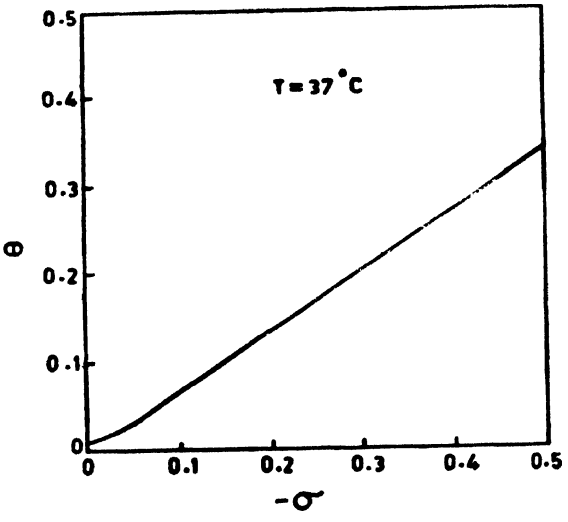

Figure 3. Supercoil-induced DNA melting at 37 C indicating substantial denaturation at highly negative supercoil densities under physiological conditions.

agreement was found to be satisfactory. Comparing our results with those for the linear form, it was found that the closed circular molecule has a much higher melting temperature T_m and a much less cooperative melting profile.

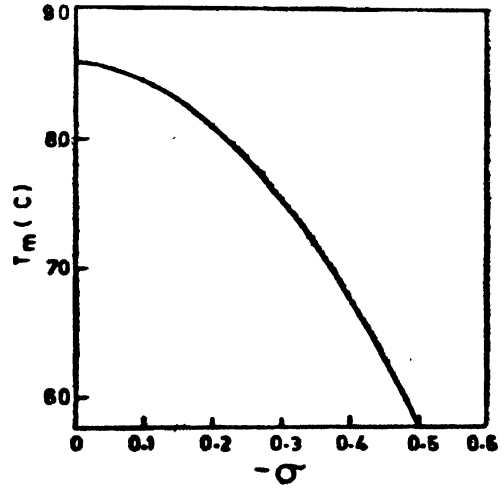

Figure 4. Lowering of melting temperature T_m in the thermal denaturation of highly supercoiled DNA.

In the present work, however, we solve the eq. (23) iteratively to obtain the supercoil-induced denaturation curve (θ vs σ) at $T = 37$°C as shown in Figure 3.

The average values of the parameters used are $C_B = 5.8 \times 10^{-19}$ erg/rad^2, $\alpha = C_B/C_Q = 23.4$, $\epsilon = 7.9$ kcal/mole and $\epsilon_0 = 2.5$ kcal/mole. Figure 3 shows that at $\sigma = -0.05$, which is the order of supercoil density for the naturally occurring plasmid DNA molecules, there is only a small denatured region present. However, at highly negative supercoil densities, we predict from Figure 3 that there should be a substantial rise in the number of denatured base pairs at the physiological temperature. This leads us to suggest that a high degree of supercoiling may facilitate the binding of other molecules to the DNA. Further, on extending our earlier work (Sen and Majumdar 1988) to compute the melting temperatures for highly negative supercoil densities, we find from Figure 4 that T_m, which has its maximum at 86°C for the relaxed closed circular form ($\sigma = 0$), decreases substantially and, in principle, becomes equal to that of the linear form, namely 58°C, for $\sigma = -0.5$, if attainable. The biological significance of these results are being investigated and further details, in this connection, will be presented in a future communication.

6. Conclusions

A general theoretical scheme for investigating the nature of different types of supercoil-induced conformational changes in a covalently closed circular B DNA molecule has been presented. We have considered the B-Z transition in a purine-pyrimidine sequence, the transition to a cruciform state in an inverted repeat sequence, and finally the melting transition of a negatively supercoiled DNA. In the case of B-Z or cruciform transition at the physiological temperature ($T = 37$°C), we predict a cooperative effect at the critical supercoil density $\sigma \simeq -0.05$, which agrees very well with the available experimental data for some specific plasmid DNA molecules with appropriate base sequences. For the supercoil-induced melting, however, we predict a continuous enhancement of the denatured region with negative supercoiling at $T = 37$°C, and the effect becomes quite substantial at highly negative supercoil densities, suggesting that this would lead to a possible mechanism of binding of the other molecules to DNA under physiological conditions. We suggest that this aspect of our results may be subjected to experimental verification. It is also found that the thermal denaturation of a closed circular DNA is, in general, facilitated by the presence of supercoiling as it reduces the melting point as well.

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